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THE INDIVIDUALITY OF THE GERM-NUCLEI DURING THE CLEAVAGE OF THE EGG OF CRYPTOBRANCHUS ALLEGHENIENSIS.

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Early observers of the process of fertilization described the meeting of the sperm-nucleus and the egg-nucleus, and their complete fusion to form a single zygotic nucleus, the primary nucleus of the embryo. Later it was found that in many cases, though apparently not in all, the two germ-nuclei merely become apposed without fusion. In tracing their further history it was shown, in certain cases, that the germ-nuclei give rise to two independent groups of chromosomes which separately enter the equatorial plate and whose descendants pass separately into the daughter-nuclei. "Later observations have given the strongest reasons for believing that, as far as the chromatin is concerned, a true fusion of the nuclei never takes place during fertilization, and that paternal and maternal chromatin *may* remain separate and distinct in the later stages of development—possibly throughout life" (Wilson, 1900, p. 204). At the present time there is much to warrant the belief that a fusion of maternal and paternal chromatin never takes place in the somatic cells, while in the lineage of germ cells nothing approaching fusion occurs until a pairing of maternal and paternal chromosomes, called synapsis, takes place in preparation for the maturation divisions; then for the first time maternal and paternal chromosomes are brought together in intimate and orderly union, in some cases amounting to fusion.

This conclusion, which is of the most fundamental importance since it vitally concerns the mechanism of heredity, is based largely on indirect evidence; for in almost every case apparently insuperable obstacles have been encountered in the attempt to trace the respective maternal and paternal chromatin-complexes through the resting stage of the nuclear cycle. In only a very

few cases has the individuality of the germ-nuclei during this stage been demonstrated with any considerable success; the observations of Häcker (1892), Rückert (1895) and Conklin (1901) are of most importance in this connection and will be discussed later.

In the developing egg of the amphibian *Cryptobranchus allegheniensis* I have found material very favorable for the study of this problem. The early cleavage nuclei are invariably double throughout the entire resting stage, each consisting of two separate though closely apposed nuclear vesicles, and the origin of each vesicle from a single germ-nucleus has been clearly traced. Indeed, my observations indicate that typically the germ-nuclei maintain their individuality throughout cleavage, though certain irregularities, real or apparent, occur with increasing frequency in the later segmentation stages.

TECHNIQUE.

Most of the sections used in making the observations recorded in the present paper were cut from eggs killed with bichromate-acetic-formalin. This mixture, made up in the right proportions (Smith, 1912, I.), preserves the form of the egg perfectly in all stages, enables it to be sectioned by the paraffin method, and gives very satisfactory histological and cytological results. For comparison, sections were made of a few eggs killed in Zenker's fluid, also in corrosive-acetic-formalin. These mixtures, particularly Zenker's fluid, gave excellent results in the fertilization and early cleavage stages, but in the blastula and gastrula stages their use is not advisable since the roof of the blastocœle or gastrocœle collapses during the process of infiltration with paraffin.

Most of the observations were made on sections of eggs stained *in toto* with Grenacher's borax-carmin, differentiated in acid alcohol, and counterstained on the slide with a mixture of Lyons blue and picric acid. This treatment leaves the chromatin stained red, the yolk granules green, the centrospheres and cytoplasmic structures blue. The method has the advantage of rapidity of manipulation, an important consideration since a

large number of slides are required. For the main purposes of this investigation the differentiation obtained by this method, used with care, is entirely adequate, but as a precaution against possible inaccuracy a parallel series was stained with Heidenhain's iron hæmatoxylin, which as might be expected gave better preparations, and is especially useful for the study of chromosomal vesicles.

All the figures excepting those of Plate IX. were outlined by means of a camera lucida and drawn with a magnification of about 600 diameters; in the process of reproduction they were reduced one third, and thus appear with a magnification of about 400 diameters. In all drawings of horizontal sections the orientation with reference to the plane of first cleavage is the same, and conforms to the diagrams in Plate IX.

In most cases the double nature of the cleavage nuclei appears to best advantage in horizontal sections, and might readily be overlooked were observations confined to vertical sections. It seems likely that most investigators studying the amphibian egg have confined their observations to vertical sections, since these give a better idea of the gross structure; and this circumstance leads us to suspect that conditions such as are here described for *Cryptobranchus* may occur in other amphibians and need only to be revealed by the study of horizontal sections.

OBSERVATIONS.

The early history of the fertilization stage of *Cryptobranchus allegheniensis* has been given in a previous paper (Smith, 1912, I.). The superficial cleavage pattern has been described in another contribution (Smith, 1912, II.).

At the time of the meeting of the germ-nuclei it is usually impossible to tell with certainty which is the egg-nucleus and which is the sperm-nucleus, but this circumstance does not affect the validity of the results recorded in this paper. The germ-nuclei do not fuse, but come to lie side by side with nuclear membranes intact (Figs. 1-4). During the long resting stage that precedes the formation of the first cleavage spindle, each germ-nucleus maintains strict individuality: there is close asso-

ciation, but no actual union, and certainly no mingling of structural contents. Hence, although it is convenient to employ such terms as "union of the germ-nuclei," and to speak of the "cleavage-nucleus" or "segmentation-nucleus" as if it represented a single entity, it should be understood that such expressions are loosely used, and the word "fusion" in this particular case is altogether inapplicable. It is the purpose of this paper to show that this unity or individuality of the respective germ-nuclei is not confined to the fertilization stage, but persists with great regularity during early cleavage, and with some apparent irregularities throughout the later cleavage and even into the gastrula stage.

The length of time intervening between the meeting of the germ-nuclei and the rupture of their nuclear membranes during the formation of the first cleavage spindle must be considerable, for resting germ-nuclei have been observed in eggs varying in age from twelve to twenty-three hours after fertilization. The precise time of formation of the first cleavage spindle has been determined in only one egg, which was killed twenty-six hours after artificial fertilization.

Since the germ-nuclei usually come to lie in the same horizontal plane, the most fruitful observations are obtained through the study of horizontal sections. The two asters, formed by the division of the single aster accompanying the sperm-nucleus on its journey to meet the egg-nucleus, take up positions on opposite sides of the resting germ-nuclei, in the same horizontal plane, and close to the region of contact of the germ-nuclei (Figs. 3 and 4). The initiation of the process of mitosis as evidenced by the appearance of distinct chromosomes and the rupture of the nuclear membranes does not take place in the two germ-nuclei simultaneously; one of the germ-nuclei becomes active somewhat in advance of the other (Figs. 5 and 6), thus furnishing evidence of some degree of physiological as well as structural independence.

In the fully-formed mitotic figure the two groups of chromosomes, of maternal and paternal origin respectively, remain visibly distinct (Fig. 7). This has been determined beyond a

doubt in a number of cases, with no conflicting evidence. In the anaphase each of the original chromosome groups has divided equally (through the splitting of individual chromosomes), the two halves going to different poles of the spindle. Each daughter-nucleus thus receives two distinct groups of chromosomes, derived from the egg-nucleus and the sperm-nucleus respectively. Although in the anaphase and early telophase (Fig. 8) the paired groups of chromosomes are not always very sharply separated, yet there is no mingling of maternal and paternal chromosomes. Finally, in the newly-formed daughter-nucleus each parental group of chromosomes becomes metamorphosed into a separate nuclear vesicle (Figs. 9 and 10). Except for a diminution in size, which is afterwards partially made good by growth, each daughter-nucleus closely simulates the original cleavage-nucleus with its two resting germ-nuclei.

The precise stage with newly-formed vesicular daughter-nuclei has not been found in horizontal sections, hence all the figures of this stage are taken from vertical sections (Figs. 9-12). During the late phases of mitosis the two daughter-nuclei move some distance apart; their path of separation is marked by a broad trail of cytoplasm sharply defined from the surrounding yolk. The division of the centrosphere or "attraction sphere" takes place shortly before the daughter-nucleus assumes the vesicular condition. The first cleavage furrow is not yet formed, but its position, in a plane at right angles to the path of separation of the two daughter-nuclei and midway between them, is visibly indicated by a condensation of cytoplasm in the blastodisc.

Aside from the fact that the two nuclear vesicles of a newly-formed daughter-nucleus are always situated approximately equidistant from the plane of first cleavage, the orientation of these nuclear vesicles with reference to each other is decidedly variable. Since the two original germ-nuclei at the time of meeting usually lie in the same horizontal plane (Figs. 2-4), we should expect the same thing of the two vesicles of a daughter-nucleus; but in the preparations studied these were more often found to lie in a plane oblique to the horizontal, and sometimes one vesicle lies directly above the other (Figs. 9 and 10). It should be remembered,

however, that the original germ-nuclei do not always lie in the same horizontal plane (Fig. 1). Moreover, the stage with newly-formed daughter-nuclei was not represented in horizontal sections, which are naturally most favorable for the discovery of nuclei consisting of vesicles lying in the same horizontal plane. There is no external landmark by means of which this stage may be recognized, and it is passed through very rapidly; in the making of horizontal sections the available material was exhausted without finding the precise stage desired.

In the study of the subsequent behavior of the nuclei the early cleavage furrows serve as convenient landmarks for orientation, and it is necessary to keep in mind the elementary fact that the second cleavage furrow forms at right angles to the first, and that a similar alternation in the direction of successive cleavage furrows is characteristic of the entire process of cleavage. Of more immediate importance to us is the correlated fact that the second nuclear division occurs in a direction at right angles to the first nuclear division, and that this alternation in the direction of successive nuclear divisions is repeated throughout cleavage. To be sure, this is strictly true only in the early cleavage stages, for there is increasing irregularity in the direction of both cleavage furrows and nuclear divisions throughout the later stages of cleavage. Incidentally, it should be noted that nuclear division always precedes the formation of the corresponding cleavage furrow.

In preparation for the second nuclear division, the two asters of a single daughter-nucleus take up positions on opposite sides of the nucleus, in the same horizontal plane and with a line connecting them parallel to the first cleavage furrow which is now usually in process of formation (Figs. 12-20; 23-26). In case the two nuclear vesicles of a single daughter-nucleus are not already ranged midway between the two asters, they rotate to this position (compare Figs. 15-16 with Figs. 17-18). Typically, the two nuclear vesicles come to lie in the same horizontal plane (Figs. 17-20, 23-26); but this is not the invariable position, for in three different eggs they were found to lie one above the other, as exemplified in Figs. 21 and 22. In any case the final

position assumed insures an equal division of maternal and paternal chromatin-groups in the ensuing mitosis. Thus the second cleavage mitosis (Figs. 27 and 28) is in all essential respects a repetition of the first, though the direction of the spindle is different. Throughout the entire segmentation period there is nothing in the behavior of the nuclei more impressive than the precision and regularity with which the two nuclear vesicles of a single nucleus are ranged side by side in the plane of the equatorial plate in preparation for the formation of the spindle.

In the resting stage following the second mitosis the two nuclear vesicles of each nucleus usually lie in the same horizontal plane (Figs. 29, 30, 32); their entire orientation indicates their derivation from the two groups of chromosomes at one pole of the spindle in the anaphase and early telophase of the preceding mitosis (Fig. 28). Occasionally, though not so often as in the preceding resting stage, one nuclear vesicle is found situated above the other (Fig. 31).

In *Cryptobranchus* the third cleavage furrows are vertical and intersect the second furrows at some distance from the first (Plate IX., Fig. 45, *J*). Thus the third cleavage furrows are nearly parallel to the first, but diverge slightly from it.

In preparation for the third cleavage mitosis the two asters take up positions on opposite sides of the nucleus, in such a situation that a line connecting them is nearly parallel to the plane of second cleavage (Figs. 33, 34; Plate IX., Fig. 45, *H*). In all cases the two nuclear vesicles entering the mitotic figure are ranged equidistant from the two asters (Figs. 33, 34, 35), and in a slightly earlier stage may be found rotating to this position (Fig. 32). Thus in the third cleavage mitosis (Fig. 36) the segregation and equal division of maternal and paternal chromosome groups is maintained. The direction of the axis of the spindle is at right angles to the preceding mitosis, and parallel to the first (Plate IX., Fig. 45, *H* and *I*). Upon the completion of mitosis the third cleavage furrows form as described in the preceding paragraph. At this time the four nuclei on the same side of the second cleavage furrow sometimes lie precisely in

the same vertical plane, so that they are all visible in a single section taken parallel to the second cleavage furrow.

The fourth cleavage furrows combine to form a circular, or more often oval, figure with its longer axis coinciding with the plane of second cleavage. This circular cleavage divides the egg very unequally, cutting off eight micromeres from eight comparatively large macromeres. Preparation for this cleavage involves rotation of the two nuclear vesicles of a single daughter-nucleus of the third cell division from their original position as shown in Fig. 37 to the position shown in Fig. 38. This latter orientation insures the maintenance of the segregation of the germ-nuclei in the fourth mitosis (Figs. 39-42). As in the previous divisions, the maternal and paternal chromosome groups have here been traced through the various phases of mitosis to the late anaphase and early telophase, where they are still distinctly separate. The position of the vesicles of the newly-formed daughter-nucleus (Figs. 43 and 44) indicates that one is of maternal origin, the other paternal. The results as regards the orientation of the nuclei up to this point are summarized in the diagrams of Plate IX., Fig. 45.

In the later cleavage divisions there is increasing irregularity in the direction of spindle axes and cleavage furrows, but this does not constitute any serious obstacle to tracing the history of the germ-nuclei since the really important point is the orientation of the nuclear vesicles with respect to the axis of the spindle. As already indicated, this is invariably such as to insure the segregation and equal representation of maternal and paternal chromosome groups in the daughter-nuclei. As the nuclei become smaller, it sometimes is difficult to distinguish the two groups of chromosomes in the late phases of mitosis, but comparison with slightly earlier and later phases leaves no doubt as to the continuity of the respective germ-nuclei. Until an advanced cleavage stage, the double structure of the resting nuclei is almost always clearly demonstrable. As the nuclei become more numerous, it becomes easier to find nuclei cut in such a manner as to show distinctly the double structure. In the more active regions of the egg, the synchronism of division

of the different cells is maintained to the late blastula stage, so that there is little likelihood of confusing cell-generations. Between micromeres and macromeres there is a fine gradation in phases of the nuclear cycle, so that a close series may be readily obtained. The individuality of the germ-nuclei has been traced without a break to an advanced cleavage stage, and it would not be difficult to illustrate this entire result with an adequate series of figures, but such a procedure would require an enormous amount of time and unduly extend the limits of this paper.

Beginning with about the seventh cell generation certain irregularities, real or apparent, occur with increasing frequency to mar the mechanical precision of the events thus far described in the history of the germ-nuclei. In the resting stage the two nuclear vesicles of a single nucleus are often deeply lobed, sometimes in such a manner as to give the impression that the nucleus is made up of many vesicles. In the late telophases the nucleus is often really made up of several or many separate though closely aggregated vesicles, but this is undoubtedly a part of the usual process of mitosis, as a more searching examination of these late phases throughout cleavage will readily show.

The transformation of the daughter-nucleus into the vesicular condition must take place with considerable rapidity, especially in the early cleavage stages, for some phases of this process have not been observed earlier than the fourth mitosis, and only occasionally in the fourth and fifth mitoses. In more advanced cleavage there is little difficulty in finding material representing the entire history of the telophase.

In the early anaphase, the long thread-like chromosomes are V-shaped, with the limbs of the V almost parallel and very straight; the apex is directed away from the equatorial plate. In a slightly later phase, which we may call the late anaphase, the chromosomes are still roughly V-shaped but finely undulating except at their free ends. A little later (early telophase) each chromosome becomes much convoluted, often coiled in a loose irregular spiral; this change begins at the apex and proceeds toward the free ends. The next step consists of the metamorphosis of each chromosome into a chromosomal vesicle, a

process which I have been able to follow in considerable detail, but will not attempt to describe here. About this time the centrosphere or "attraction sphere" divides.

During the anaphase and early telophase the maternal and paternal chromosome groups are almost always clearly separated, sometimes with a broad space between the two groups. The segregation of the chromosomal vesicles into two groups, maternal and paternal respectively, is usually evident. In the late telophase the chromosomal vesicles of each group unite to form separate nuclear vesicles which for a time show indications of their manner of origin through the persistence of lobes separated by deep clefts. The inner boundaries of the chromosomal vesicles persist for a time as partitions in the nuclear vesicles, but finally disappear wholly or in part. In the late blastula the vesicular nucleus in many cases retains a lobed structure throughout the resting stage. It is possible that in some cases the fusion of chromosomal vesicles to form vesicular nuclei of the usual duplex type is never completed, for in the late blastula and early gastrula nuclei consisting of three, four or even more well-rounded vesicles are not uncommon.

How much longer and to what extent during the development of the embryo a strict separation of the germ-nuclei is maintained I have not attempted to determine, but it is not necessary to conclude that it continues throughout the life of the organism, either in the somatic or the germ cells. In the discussion it will be shown that the individuality of the germ-nuclei is not incompatible with a mingling of maternal and paternal chromosomes, and there is no biological necessity for a strict separation of the germ-nuclei in any stage from fertilization to synapsis.

DISCUSSION.

In 1875 Oscar Hertwig and Hermann Fol showed that the fertilized egg contains two nuclei, one belonging to the egg itself and the other introduced by the spermatozoön. While the earliest observers of the process of fertilization, notably Auerbach, Strasburger and Hertwig, described the complete fusion of these germ-nuclei to form the first embryonic nucleus, called by

Hertwig the cleavage-nucleus or segmentation-nucleus, later observations showed that such is not always the case. In 1881 Mark demonstrated that in the slug *Limax* the two germ-nuclei do not fuse; after coming together they persist during the formation of the two cleavage centers, then their membranes gradually disappear. Two years later Van Beneden showed that in *Ascaris*, not only do the germ-nuclei become apposed without fusion, but each gives rise to an independent group of chromosomes which contribute equally to the formation of the daughter-nuclei. Thus the foundation of the doctrine of the biparental character of the nuclei of sexually-produced organisms was laid down by Van Beneden. In many animals, and in some plants (gymnosperms), the independence of the formation of the maternal and the paternal chromosome groups following fertilization has been established by direct observation, though the demonstration has seldom been carried beyond the first cleavage stage. On the other hand there are some animals, and many plants, in which the germ-nuclei meet and fuse while in the resting condition, so that in the chromatin of the resulting nucleus maternal and paternal contributions cannot be readily distinguished.

Häcker (1892 and 1895) and Rückert (1895) found that the germ-nuclei of *Cyclops* do not fuse but preserve their individuality throughout at least a considerable period of the cleavage of the egg. In mitosis the two groups of chromosomes, of maternal and paternal origin respectively, remain distinct and bilaterally distributed, while each resultant daughter-nucleus in the resting stage consists of two closely apposed but structurally separate vesicles.

In 1901 Conklin described the double structure of the cleavage nuclei of *Crepidula* in certain stages of the nuclear cycle, and pointed out that the two halves which at times appear as distinct entities are almost certainly to be regarded as of maternal and paternal origin respectively. "This separateness is most easily observed in the telophase of each division, though in some cleavage cells it may be seen in the prophase also, or even throughout the resting period. At the time when the daughter-nuclei are being formed the chromosomal vesicles fuse into two groups

which are closely pressed together but still separated by a partition wall, as Rückert has shown to be the case in *Cyclops*. Gradually this partition wall disappears, being preserved longest on that side of the nucleus nearest the centrosome. Here a groove is formed on one side of the nucleus which marks the line of contact between the two halves. In some cleavage cells this groove is visible throughout most of the resting period; in others it disappears during the greater part of the resting period, though it may reappear in the following prophase; in all cases, however, the partition wall and groove reappear in the next succeeding telophase, when it is again formed in the manner described above. I have observed the double character of the nucleus in the telophase of every cleavage up to the 29-cell stage, and in several of the later cleavages up to the 60-cell stage, though it becomes more difficult to see as the nuclei grow smaller. . . . It still remains to show that these double nuclei really represent the egg and sperm nuclei which have not yet lost their individuality. This cannot be demonstrated in *Crepidula*, for the reason that this double character is not apparent at every stage in the nuclear cycle, but it is extremely probable" (Conklin, 1901). Additional observations recorded by Conklin in support of his interpretation may be summarized as follows: (1) In the metaphase of the first cleavage division the maternal and paternal germ-nuclei are represented by separate groups of chromosomes; in the early anaphase these groups of chromosomes can no longer be distinguished, but the nuclei are clearly double in the immediately following late anaphase and telophase, and the position of the partition wall in these double nuclei corresponds to the plane of contact between the germ-nuclei. (2) The groove which is found on one side of the nucleus in the telophase of the first cleavage mitosis persists well into the resting stage, and a corresponding groove is found in the same position in the prophase of the second mitosis. The central spindle for the second cleavage mitosis lies in this groove, and thus the amphiaster actually lies in the only plane in which it would be possible to halve the two parts of the double nuclei. Although cleavage divisions successively alternate in direction, unequal division of the double nuclei is

prevented either by a rotation of the nucleus during the resting stage, or by a rotation of the spindle in the early stage of mitosis.

(3) In certain abnormal cleavages the double nuclei are really two entirely separate nuclei lying side by side within a single cell.

(4) In each of the germ-nuclei, before they come into contact, there is a single nucleolus; these nucleoli disappear in the prophase of the first cleavage, but in the succeeding telophase a single nucleolus generally appears in each half of each daughter-nucleus. The same is true of the succeeding cleavages, so that each nucleus throughout the cleavage usually has two nucleoli in the telophase or early resting stage.

Beard (1902) described a double structure of the resting stages of the nuclei of the early germ cells of *Raja batis*; these were not traced earlier than a late gastrula stage, but influenced by the findings of Häcker and Rückert, Beard did not hesitate to interpret the double nuclei as consisting of distinct maternal and paternal halves.

Jenkinson (1904) gives some interesting figures of the fertilization and first cleavage stages of *Axolotl*. The germ nuclei meet without fusion, and the chromosomes appear separately in each pronucleus while the nuclear membranes are still intact. In some cases at least, these two chromosome groups remain distinct in the equatorial plate after the dissolution of the nuclear membranes. Scant attention is paid to these features in the text of Jenkinson's paper, which is concerned with other matters, but the author states that he has found two distinct sets of chromosomes in some preparations of the fertilization spindle of *Triton*.

In 1904 Moenckhaus described the independence of the maternal and paternal chromosome groups in the early cleavage spindles of the hybrids produced by fertilizing the eggs of *Fundulus* with *Menidia* sperm. The difference in the size and shape of the chromosomes of the two species makes the identification of the maternal and paternal chromosomes in the case described a matter of certainty.

Pinney (1918) found that two nucleoli are typically present in the nuclei of normal *Ctenolabrus* blastoderms, and cited evi-

dence supporting the view that in these double nucleoli observed in fishes we are dealing with parental homologues.

Concerning *Cryptobranchus allegheniensis*, the writer believes that the observations recorded in the present paper establish beyond question the complete separation of maternal and paternal germ-nuclei to a late blastula stage at least. The separation is particularly marked during the resting stage of the nuclear cycle, precisely where most investigators working with other species have encountered the greatest difficulty.

The observations thus far cited indicate that in certain forms the individuality of the germ-nuclei during early embryonic development is maintained by complete separation of the nuclear material derived from the egg and the spermatozoon respectively. It has already been indicated that this segregation is not by any means a universal phenomenon. Is it possible that the individuality of the germ-nuclei may be maintained, in all essential respects, in those other cases where there is a mingling of chromatin derived from the two germ-nuclei? Let us first examine the facts that require explanation.

In all cases where the germ-nuclei fuse into a single vesicular nucleus before the formation of the first cleavage spindle, mingling of maternal and paternal chromosomes may be expected. Sax (1918) has recently described two cases in flowering plants, *Fritillaria* and *Triticum*. In *Fritillaria* the germ-nuclei usually unite while in the resting condition, although occasionally they are in the spireme stage at the time of fusion. The presence of a single spireme in the zygote could not be demonstrated. In *Triticum* the sperm-nucleus is small and almost homogeneous in structure even while in contact with the egg nucleus. The sperm-nucleus enters the egg-nucleus and there forms a separate compact spireme; at the same time the spireme of the egg-nucleus is formed. In both *Fritillaria* and *Triticum* the maternal and paternal chromosomes are formed independently, but they are not found in separate groups.

Moenckhaus (1904) found that after the first few cleavage divisions in his hybrid teleost eggs the chromosomes of maternal and paternal origin mingled indiscriminately upon the equatorial

plate, and the observations of Morris (1914) are in agreement.

Metz (1916) has found in the diptera a pairing of homologous chromosomes, simulating synapsis, which occurs in all tissues, somatic as well as germinal; this association of maternal with paternal chromosomes was found in late cleavage and during all later stages of embryonic development. From the following statement by Overton (1909) we have a suggestion of a similar occurrence in plants, though attention is directed more particularly to the matter of genetic continuity of individual chromosomes: "In the somatic nuclei (of certain plants) the chromosomes are represented during rest by definite visible bodies, the pro-chromosomes, which are arranged in parallel pairs, with apparent linin intervals. These heterogeneous spirems, the homologous portions of which have early become associated in pairs, probably remain distinct throughout the life-history of the sporophyte."

In spite of the mingling and even paired association of maternal and paternal chromosomes, there are reasons for believing that the two kinds of chromosomes maintain their independence until gametogenesis. This leads us to a consideration of the doctrine of the genetic continuity of individual chromosomes, which goes further than the principle of duality of the embryonic nuclei, but confirms it as a universal law.

The remarkable constancy in the number of chromosomes throughout the cells of a given organism and species has long been known, and affords important evidence for the view that the chromosomes are persistent as individual structures. To be sure, it sometimes happens in mitosis that one or more chromosomes belonging to one daughter-group, accidentally become included with the other group so that one of the daughter-nuclei has fewer, the other more, than the normal somatic number; but such an occurrence is very exceptional, and in subsequent divisions of these cells the number of chromosomes appearing is not the normal, but the increased or diminished number (Boveri, 1890). Whatever the number of chromosomes entering into the formation of a resting nucleus, the same number afterwards issues from it.

In 1883 Van Beneden showed that in *Ascaris* the spermatozoön brings in just as many chromosomes as are contained in the egg. As a result of a careful study of mitosis in epithelial cells of the salamander, Rabl (1885) concluded that the chromosomes do not lose their individuality at the close of division, but persist in the chromatic reticulum of the resting nucleus. Boveri (1887 and 1888) supported Rabl's hypothesis on the ground of his own studies and those of Van Beneden on the early stages of *Ascaris*. Boveri demonstrated in *Ascaris* that in the formation of the spireme the chromosomes reappear in the same position as those which entered into the formation of the reticulum, precisely as Rabl had maintained. As the long chromosomes diverge, their free ends are always turned toward the equator of the spindle, and upon the reconstruction of the daughter-nuclei these ends give rise to corresponding lobes of the nucleus, which persist throughout the resting stage. At the succeeding division the chromosomes reappear exactly in the same position, their ends lying in the nuclear lobes as before. These observations were afterwards confirmed by Herla (1893), and more recently Sutton (1902) has observed practically the same thing in *Brachystola magna*. Boveri (1891) concluded that the chromosomes must be regarded as individuals that have an independent existence in the cell, and expressed his belief that "we may identify every chromatic element arising from a resting nucleus with a definite element that entered into the formation of that nucleus, from which the remarkable conclusion follows that in all cells derived in the regular course of division from the fertilized egg, one half of the chromosomes are of strictly paternal origin, the other half of maternal."

Herla (1893) and Zoja (1895) have shown that if in *Ascaris megalocephala* the egg of variety *bivalens*, having two chromosomes, be fertilized with the spermatozoön of variety *univalens*, having one chromosome, the three chromosomes reappear at each cleavage, at least as far as the twelve-cell stage; and according to Zoja, the paternal chromosome is distinguishable from the two maternal at each step by its smaller size. "We have thus what must be reckoned as more than a possibility, that every cell

of the body of the child may receive from each parent not only half of its chromatin substance, but one half of its chromosomes, as distinct and individual descendants of those of its parents " (Wilson, 1900).

Boveri (1909) found in *Ascaris* that in sister cells preparing for division the configuration of the groups of chromosomes is the same. The similarity of the sister cells is explicable on the view that the chromosomes retain during the resting stage the same shape and size and relative location that they had at the end of the preceding division. In cells of these same embryos that are not sister cells, a great variety of arrangements of the chromosomes is found, and no two arrangements are so nearly alike as are those found in sister cells.

Other evidence for the continuity of individual chromosomes is derived from those cases where the reconstruction of the resting nucleus takes place through the metamorphosis of each chromosome into a hollow vesicle, and the aggregation or fusion of these chromosomal vesicles to form a single nucleus. Such, to be sure, is not the only type of telophase (Wilson, 1900, p. 71), but it takes place in many segmenting ova and in some spermatogonia. According to Conklin (1902), in the late stages of mitosis of the segmenting egg of *Crepidula* the chromosomes enlarge to form vesicles and these unite into a resting nucleus; the nuclear membrane is composed of the outer walls of the vesicles, while the inner walls stretch through the nucleus as chromatic partitions; the chromosomal vesicles from the egg and sperm nuclei respectively remain distinct longer than those from the same germ nucleus. Vesicular chromosomes have been described in fish eggs by Moenckhaus; the individual vesicles fuse with their neighbors and these larger ones with each other until at last the entire nucleus is simply one great vesicle, which is at first lobed, but later is well rounded. Wenrich (1916) says of *Phrynotettix*: "The spermatogonial divisions showed that each chromosome forms a sac or vesicle in the earlier telophases, and that it expands and becomes diffused within these vesicles; that, although the vesicles appeared to coalesce, there is always a remnant of each chromosome visible in the center of the region occupied by the

vesicle, and that in the prophase the chromatin concentrates about this remnant or core and there forms a spirally coiled thread, which develops into a prophase chromosome." Richards (1917) has shown that in the cleavage and gastrula stages of *Fundulus* and *Coregonus* the chromosomes in the telophase of mitosis enlarge to form vesicles which remain distinct though compactly massed together during the resting stage. The new chromosome arises within a vesicle, through the aggregation of its granules; thus there is genetic continuity of individual chromosomes. According to Richards, the polarity of the cell is manifested in the arrangement of the elongated vesicles. When the centrosome divides, the cell acquires a new axis at right angles to the old, in a line connecting the two asters; the chromosomes, when formed, orient themselves with respect to the new axis. Pinney (1918) figures chromosomal vesicles in the telophase of dividing blastomeres of *Ctenolabrus* \times *Fundulus* hybrids. Some observations on chromosomal vesicles in *Cryptobranchus alleghehiensis* are included in the present paper. I have examined a few sections of *Coregonus* blastoderms belonging to the collection of the Michigan State Normal College and the material appears to be exceptionally favorable for the study of chromosomal vesicles.

Thus it has been proved that in the fertilized egg one half of the chromosomes are derived from the father and one half from the mother, and that at every division of the egg the chromosomes also divide in such a manner that their progeny are distributed in equal number to all the cells of the egg. Further, there is genetic continuity between each individual chromosome that enters the resting stage and a corresponding chromosome that emerges in preparation for the next division. The conclusion is thus reached that the fertilized egg, and all the cells derived from it, contain a double set of chromosomes, paternal and maternal. This conclusion implies that at every step the respective chromosome groups preserve their independence, however much they are mingled with one another.

This conclusion is further strengthened by observations of individual differences, both morphological and physiological,

between the chromosomes of a given simplex group, and by the behavior of the chromosomes in synapsis. The chromosomes of a single group, maternal or paternal, are not precisely alike, but differ among themselves in size, form and genetic potency (Montgomery, 1901; Sutton, 1902 and 1903; Morgan, 1915). In certain species the size differences are very marked, so that the chromosomes of a single germ-nucleus may be arranged in a graded series; these size differences are constant from one cell-generation to the next, so that individual chromosomes may be identified in successive cell-divisions. In other species where the visible differences are not so marked we have evidence that physiological differences exist, for Boveri (1907) has shown the strong probability that normal development of the egg is possible only in the presence of at least a single set of qualitatively different chromosomes. Thus the concept of the individuality of the chromosomes has been extended to include not merely the genetic continuity of each particular chromosome, but also its idiosyncrasy or specificity.

In any biparental organism, the duplex chromosome group is composed of two equivalent parental series or simplex groups, in which each individual chromosome is homologous with a very similar chromosome belonging to the other series; in other words, the chromosomes are present in biparental pairs (Montgomery, 1901; Sutton, 1902 and 1903; Wilson, 1912). "In the process known as synapsis, which takes place shortly before the last two cell-divisions concerned in the formation of the germ cells, the chromosomes do in fact unite in pairs, two by two. There is reason to believe that the two members of each pair are respectively of maternal and paternal derivation; and the probability of this view, first stated by Montgomery, has steadily increased." (Wilson, 1913). In one of these two final divisions of the germ cell cycle the double chromosome groups are reduced to single ones in preparation for the subsequent process of fertilization; this reduction is accomplished through the failure of the individual chromosomes to split in the process of mitosis. In this reducing division the two members of a synaptic pair are separated to pass into different daughter cells, but not necessarily

in such a manner that each daughter cell receives exclusively maternal or exclusively paternal chromosomes. The distribution of each pair of homologous chromosomes is entirely independent of that of every other pair, so that in the daughter cells combinations of non-homologous chromosomes occur regardless of their parental origin; each daughter cell usually receives a mixture of maternal and paternal chromosomes in varying proportions. Within the limits of the reduced number, any combination of the chromosomes furnished by the immature germ cell is possible in a daughter cell resulting from the reducing division, save that a single daughter cell cannot receive both members of a synaptic pair. Synapsis is thus explained by the provision which it makes that two homologous chromosomes shall in no case enter the nucleus of a single spermatozoon or mature egg (Sutton, 1902). The principle of random distribution or independent assortment of non-homologous chromosomes has been confirmed by the direct observations of Carothers (1913 and 1917), Wenrich (1915 and 1916) and others. The point that immediately concerns us here is the fact that in the reducing division the individuality of the germ-nuclei is usually lost, for it seldom happens that a single daughter cell, and consequently a single mature gamete, receives exclusively maternal or exclusively paternal chromosomes.

"Synapsis is not a haphazard junction of chromosomes, but an orderly union of elements of maternal and paternal origin, similar in size, in details of form, and probably also in function" (Kellicott, 1913, p. 294). The orderly processes of heredity as they exist today in biparental organisms would be impossible were the integrity of the maternal and paternal chromosome groups not maintained up to the time of synapsis. In *Cryptobranchus allegheniensis* and in some other forms this integrity is manifested at every step by a complete segregation of maternal and paternal chromosome groups; in other forms the independence of the chromosome groups is maintained in spite of the mingling of maternal and paternal chromosomes. The persistent individuality of the chromosomes is the important thing, and so long as this is maintained the germ-nuclei exist as actual

entities whether visibly separated or not. The distinction between the two classes of germ-nuclei—those that fuse at the time of fertilization and those that do not—is more apparent than real. The expression “individuality of the germ-nuclei” seems justified in either case, for individuality implies separableness as well as separateness (Conklin, 1916); but some may prefer to use the term “autonomy,” which places the emphasis upon independence. Since attention has become focused upon the chromosomes, the expression “autonomy of the maternal and paternal chromosome groups” has often been used to indicate the duality of nuclear structure.

Where it exists, the separation of maternal and paternal chromatin-complexes into distinct groups within a single nucleus affords a striking exemplification of the deeper and more universal truth that each germ-nucleus is represented in its entirety in every cell, somatic as well as germinal, of a developing organism. In *Cryptobranchus allegheniensis* this dual structure of the nucleus is clearly visible in every cell of the early segmentation stages at least, so that here we have material for an ocular demonstration of a principle long ago foreseen by Huxley (1878), who wrote: “It is conceivable, and indeed probable, that every part of the adult contains molecules derived from both the male and the female parent; and that, regarded as a mass of molecules, the entire organism may be compared to a web of which the warp is derived from the female and the woof from the male.”

SUMMARY.

In the fertilization of the egg of *Cryptobranchus allegheniensis* the germ-nuclei do not fuse, and in the first cleavage mitosis each gives rise to a separate group of chromosomes whose descendants pass separately to the daughter-nuclei.

During the ensuing resting stage each germ-nucleus is represented by a structurally distinct vesicle. The separateness of the germ-nuclei is thus maintained throughout the entire nuclear cycle.

Throughout early cleavage the nuclear divisions are of the same duplex type, and the resting nuclei are always distinctly

double. The genetic continuity of each half of the double nucleus has been clearly traced to an advanced cleavage stage.

During late cleavage and in the early gastrula the nuclei are still typically double, but certain irregularities which tend to disguise the double structure occur with increasing frequency and the segregation of maternal and paternal chromatin cannot always be demonstrated.

The hypothesis of individuality of the germ-nuclei as applied to those species in which there is a mingling of maternal and paternal chromosomes is discussed, and supported by considerations regarding the persistent individuality of the chromosomes.

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EXPLANATION OF PLATE I.

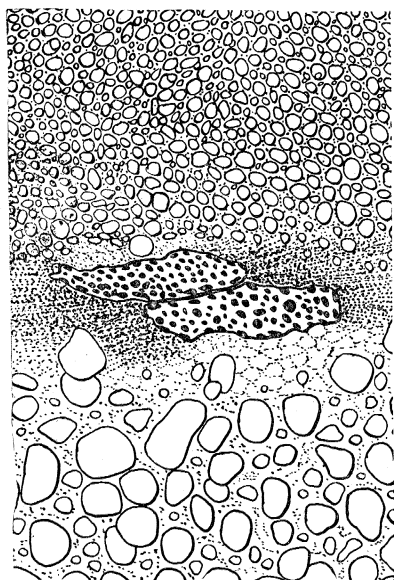
Cryptobranchus allegheniensis.

FIG. 1. Portion of a meridional section of a fertilized egg, showing the meeting of the sperm-nucleus and the egg-nucleus. Each germ-nucleus extends through four sections, of which an inner one is shown in the figure. The section passes midway between the two asters, which lie on opposite sides of the nuclei in a line at right angles to the plane of the section. The egg was fixed with bichromate-acetic formalin and stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

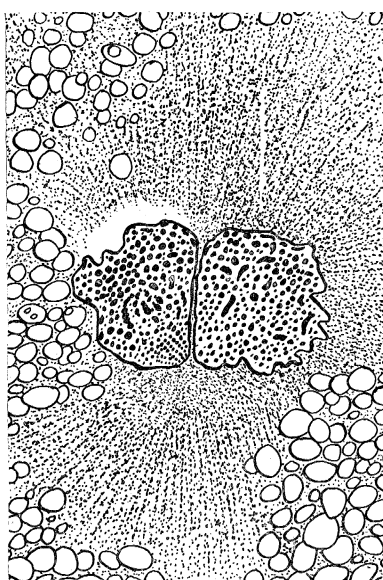
FIG. 2. Meridional section of an egg killed twenty-two and one half hours after fertilization, showing the resting germ-nuclei. Each germ-nucleus extends through five sections, of which the middle one is here shown. The section passes midway between the two asters, which lie close to the apposed surfaces of the two nuclei in a line oblique to the plane of the section. Fixed with bichromate-acetic-formalin; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

FIG. 3. Horizontal section through the resting germ-nuclei of an egg killed twenty-one and one half hours after fertilization. Nearly half of each nucleus is left in an adjacent section. Two asters are shown in the figure. Fixed with bichromate-acetic-formalin; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

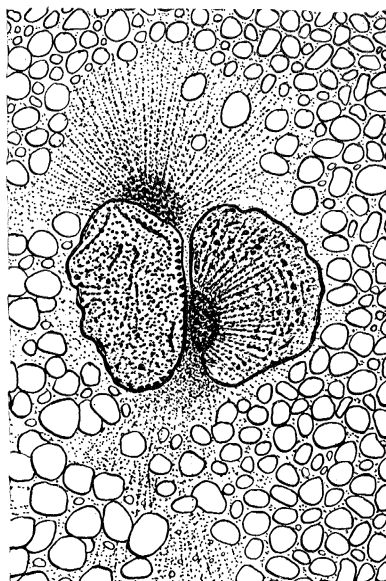
FIG. 4. Horizontal section through the resting germ-nuclei of an egg killed twenty-two and three fourths hours after fertilization. Each germ-nucleus extends through three sections, of which the middle one is here shown. Two asters are shown in the figure. Bichromate-acetic-formalin fixation; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.



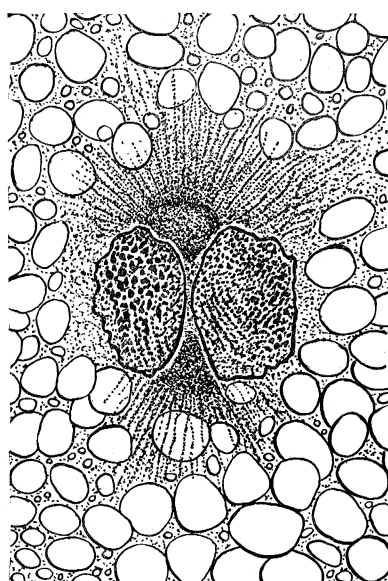
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EXPLANATION OF PLATE II

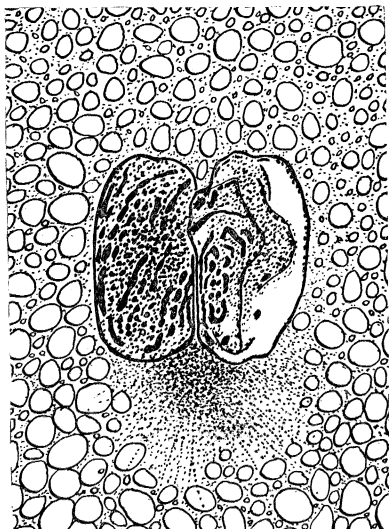
Cryptobranchus alleghehiensis.

FIG. 5. Horizontal section of a fertilized egg showing the germ-nuclei preparing for the first cleavage mitosis. The germ-nucleus shown at the right is in a slightly more advanced phase than the other. A portion of each nucleus is left in an adjacent section. Bichromate-acetic-formalin fixation; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

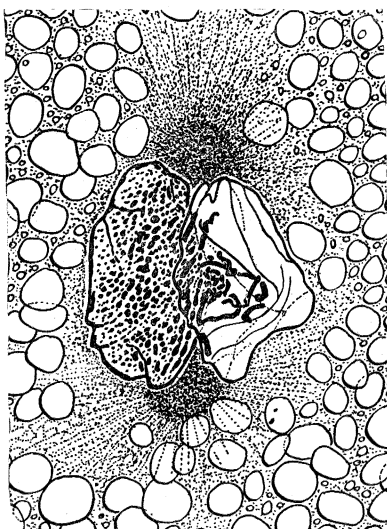
FIG. 6. Horizontal section of an egg killed twenty-two and one half hours after fertilization, showing the germ-nuclei preparing for the first cleavage mitosis. The germ-nucleus shown at the right is in a more advanced phase than the other. About half of each nucleus is left in an adjacent section. Bichromate-acetic-formalin fixation; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

FIG. 7. Horizontal section through the first cleavage spindle (late prophase). The chromosomes are segregated into two groups, of maternal and paternal origin respectively. A very small portion of each group of chromosomes is left in each of the adjacent sections. Bichromate-acetic-formalin fixation; stained with iron-hæmatoxylin. $\times 400$.

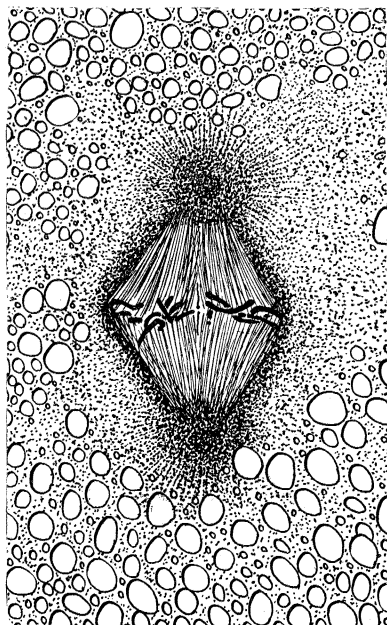
FIG. 8. Horizontal section of an egg killed twenty-six hours after fertilization, showing a late stage (early telophase) of the first nuclear division. In the distribution of chromatin to the daughter-nuclei, the segregation of maternal and paternal chromatin is maintained. A very small portion of the chromatin of each daughter-nucleus is left in an adjacent section. Bichromate-acetic-formalin fixation; stained with borax-carminc Lyons-blue picric acid. $\times 400$.



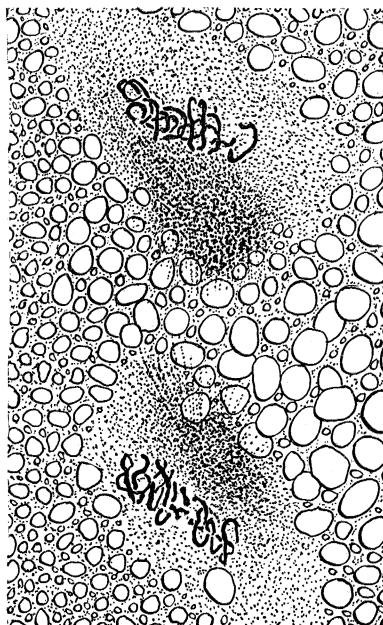
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EXPLANATION OF PLATE III.

Cryptobranchus allegheniensis.

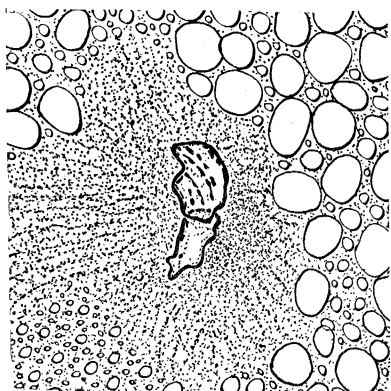
FIGS. 9 AND 10. Two successive vertical sections through a single daughter-nucleus of the first cleavage mitosis. The sections are nearly meridional, and pass very close to the other daughter-nucleus, hence at right angles to the plane of the first cleavage furrow which is not yet formed. The nucleus shown is almost entirely confined to the two sections. Bichromate-acetic-formalin fixation; stained with borax-carminc Lyons-blue picric acid. $\times 400$.

FIG. 11. Vertical section through a single daughter-nucleus of the first cleavage mitosis. The section is nearly meridional, and passes very close to the other daughter-nucleus, hence at right angles to the plane of the first cleavage furrow which is not yet formed. A considerable part of each nuclear vesicle is left in an adjacent section, and comparison with these other portions shows that the plane of contact of the two nuclear vesicles is oblique to the plane of the section. Bichromate-acetic-formalin fixation; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

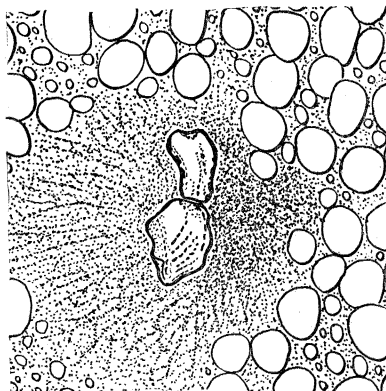
FIG. 12. Vertical section showing a single daughter-nucleus of the first cleavage mitosis, taken at right angles to the plane of the first cleavage furrow which is not yet formed. The entire double nucleus is confined to this section. Bichromate-acetic-formalin fixation; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

FIG. 13. Vertical section, parallel to the newly-formed first cleavage furrow, through a single daughter-nucleus of the first cleavage division. Part of the nucleus is left in an adjacent section, where it is likewise distinctly double. Bichromate-acetic-formalin fixation; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

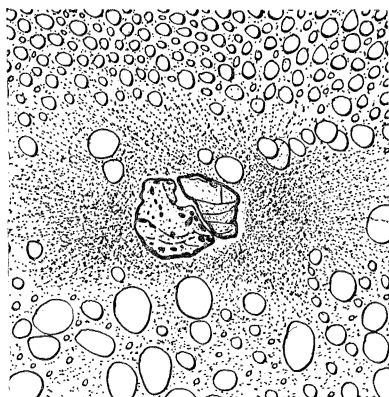
FIG. 14. Vertical section, parallel to the first cleavage furrow, through the other daughter-nucleus of the egg used for Fig. 13. Part of the nucleus is left in an adjacent section, where the two nuclear vesicles are more closely united. Bichromate-acetic-formalin fixation; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.



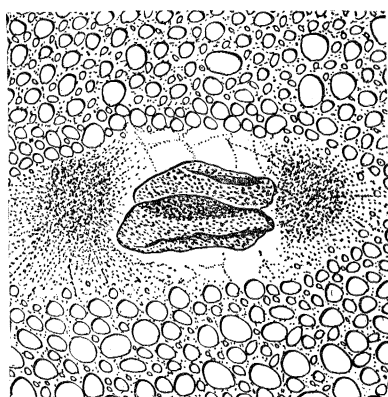
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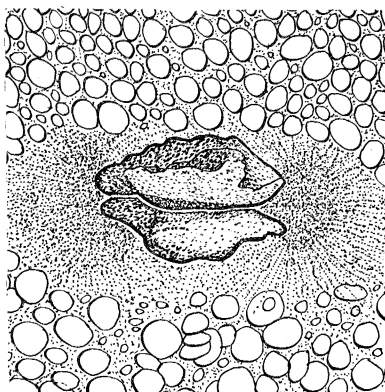
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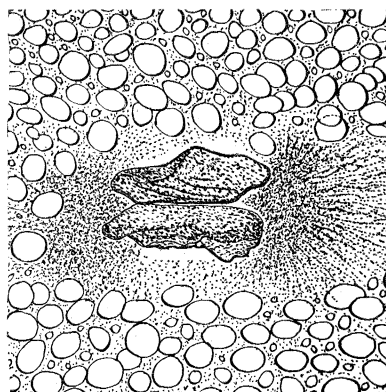
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EXPLANATION OF PLATE IV.

Cryptobranchus allegheniensis.

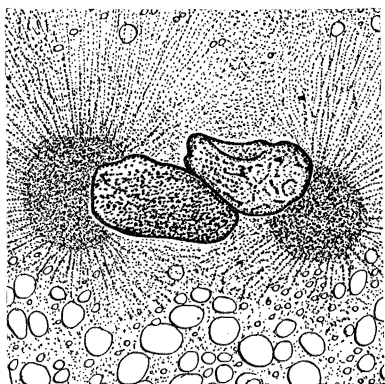
FIGS. 15 AND 16. Two successive horizontal sections showing a single nucleus rotating into position for the second cleavage mitosis; almost the entire nucleus is confined to these two sections. The newly-formed first cleavage furrow extends in a direction parallel to a line connecting the two asters. Fixed in bichromate-acetic-formalin; stained with iron-hæmatoxylin. $\times 400$.

FIG. 17. Horizontal section through a single nucleus in position for the second cleavage mitosis; the greater part of the nucleus lies in this section. The newly-formed first cleavage furrow extends in a direction parallel to a line connecting the two asters. Fixed in Zenker's fluid; stained with iron-hæmatoxylin. $\times 400$.

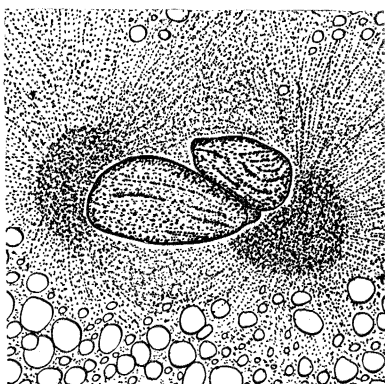
FIG. 18. Horizontal section through the other nucleus of the egg containing the nucleus shown in Fig. 17. Orientation as in the preceding figure. Stained with iron-hæmatoxylin. $\times 400$.

FIG. 19. Horizontal section showing a single nucleus in position for the second cleavage mitosis; the entire nucleus lies in this section. The newly-formed first cleavage furrow extends in a direction parallel to a line connecting the two asters. Bichromate-acetic-formalin fixation; stained with borax-carmin Lyons-blue picric-acid. $\times 400$.

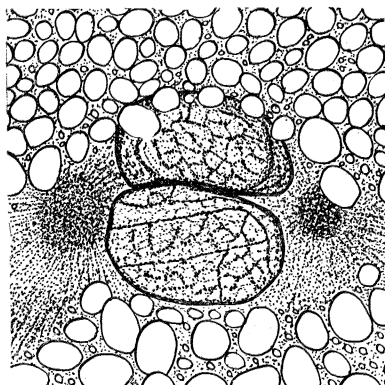
FIG. 20. Horizontal section through the other nucleus of the egg used for Fig. 19. A small part of one nuclear vesicle, and the greater part of the other, lies in an adjacent section. Orientation as in the preceding figure. Stained with borax-carmin Lyons-blue picric-acid. $\times 400$.



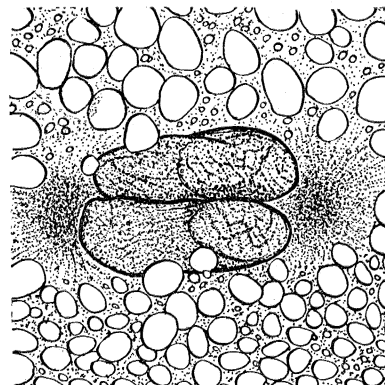
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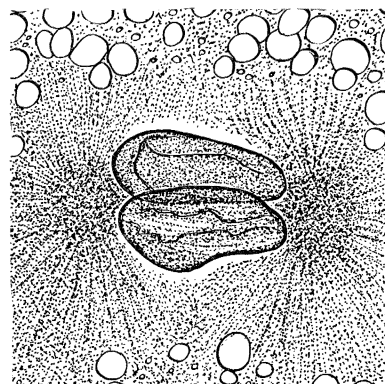
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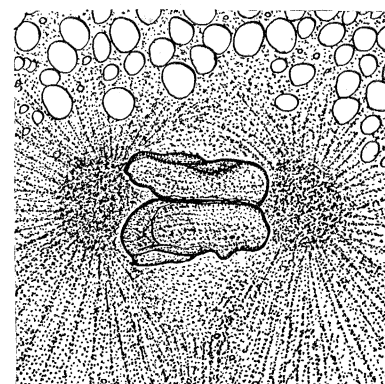
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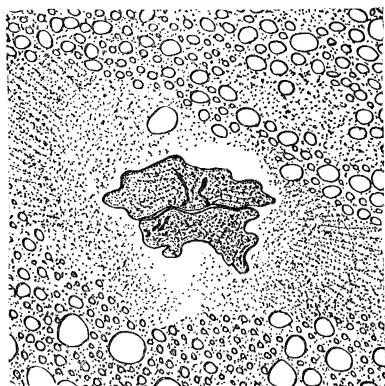
EXPLANATION OF PLATE V.

Cryptobranchus alleghehiensis.

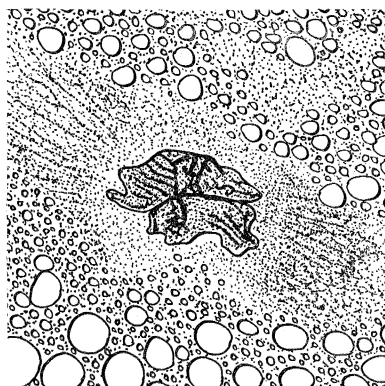
FIGS. 21 AND 22. Two successive meridional sections through a single nucleus preparing for the second cleavage mitosis. The sections extend at right angles to the first cleavage furrow, and close to the other cleavage-nucleus. A considerable part of the nucleus here shown is left in an adjacent section where it is not so clearly separated into two nuclear vesicles. Fixed in bichromate-acetic-formalin; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

FIG. 23. Horizontal section through a single nucleus preparing for the second cleavage mitosis; the nucleus is almost entirely confined to this section. The first cleavage furrow extends in a direction nearly parallel to a line connecting the two asters. Bichromate-acetic-formalin fixation; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

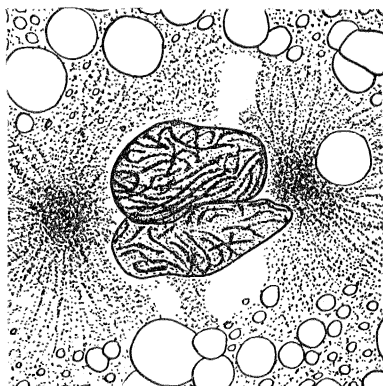
FIGS. 24, 25 AND 26. Three successive horizontal sections through a single nucleus entering upon the second cleavage mitosis; all but a very small portion of the nucleus is included in these sections. The first cleavage furrow extends in a direction nearly parallel to a line connecting the two asters. Bichromate-acetic-formalin fixation; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.



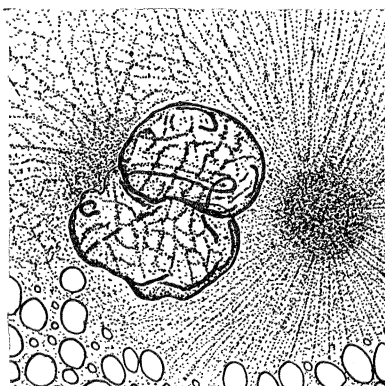
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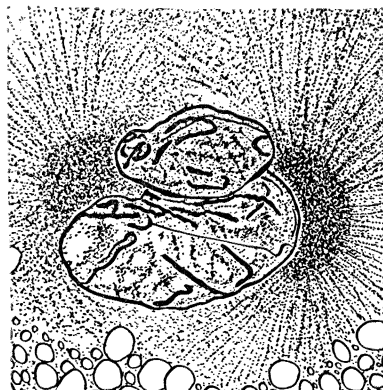
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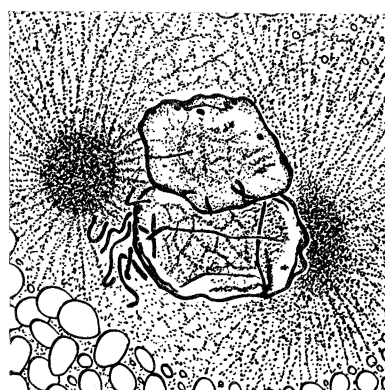
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EXPLANATION OF PLATE VI.

Cryptobranchus algehiensis.

FIG. 27. Horizontal section through a nucleus in a late prophase of the second cleavage mitosis. A small portion of each group of chromosomes is left in each of the adjacent sections. The axis of the spindle lies in a direction nearly parallel to the plane of first cleavage. Fixed with bichromate-acetic-formalin; stained with borax-carmines Lyons-blue picric-acid. $\times 400$.

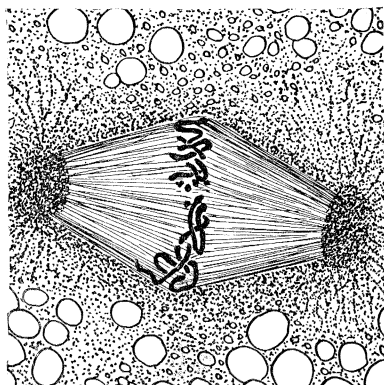
FIG. 28. Horizontal section through one of the daughter-nuclei in a late stage (early telophase) of a second cleavage mitosis. Orientation as in the preceding figure; the second cleavage furrow is not yet formed. Fixed in Zenker's fluid; stained with iron-haematoxylin. $\times 400$.

FIG. 29. Horizontal section through one of the daughter-nuclei of a second cleavage mitosis; early resting stage. Part of the nucleus is left in an adjacent section where it is likewise distinctly double. The plane of apposition of the two nuclear vesicles is parallel to the first cleavage furrow, and at right angles to the second cleavage furrow which is beginning to form. Bichromate-acetic-formalin fixation; stained with iron-haematoxylin. $\times 400$.

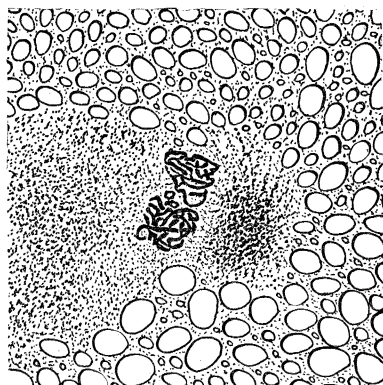
FIG. 30. Horizontal section through one of the daughter-nuclei of a second cleavage mitosis; resting stage. A portion of the nucleus is left in each of the adjacent sections where it is likewise distinctly double. The plane of apposition of the two nuclear vesicles is parallel to the plane of first cleavage, and at right angles to the beginning second cleavage furrow. Bichromate-acetic-formalin fixation; stained with borax-carmines Lyons-blue picric-acid. $\times 400$.

FIG. 31. Vertical section through one of the daughter-nuclei of a second cleavage mitosis; resting stage. The section is taken parallel to the plane of the second cleavage furrow which has just begun to form. Part of the nucleus is left in an adjacent section, where it is likewise distinctly double. Bichromate-acetic-formalin fixation; stained with borax-carmines Lyons-blue picric-acid. $\times 400$.

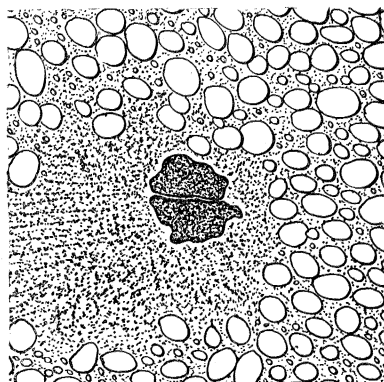
FIG. 32. Horizontal section through a nucleus rotating into position for the third cleavage mitosis; almost the entire nucleus is confined to this section. The second cleavage furrows are present and extend nearly to the equator of the egg. Bichromate-acetic-formalin fixation; stained with borax-carmines Lyons-blue picric-acid. $\times 400$.



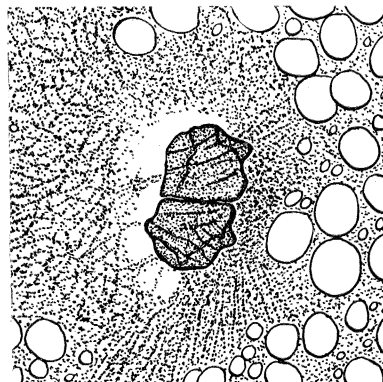
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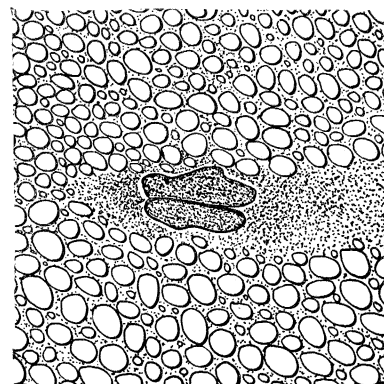
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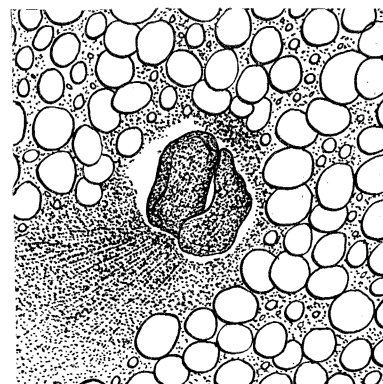
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EXPLANATION OF PLATE VII.

Cryptobranchus allegheniensis.

FIG. 33. Horizontal section through a resting nucleus in position for the third cleavage mitosis. The plane of apposition of the two nuclear vesicles is nearly parallel to the *second* cleavage furrows. A considerable part of the nucleus is left in an adjacent section, where it is likewise distinctly double. Fixed with bichromate-acetic-formalin; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

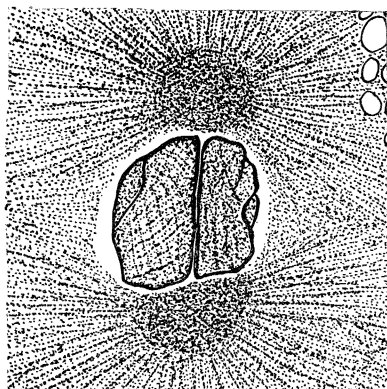
FIG. 34. Horizontal section through a nucleus in position for the third cleavage mitosis. A line connecting the two asters is nearly parallel to the second cleavage furrow. Part of the nucleus is left in an adjacent section, where it is likewise distinctly double. Fixed with bichromate-acetic-formalin; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

FIG. 35. Vertical section through a nucleus entering upon the third cleavage mitosis. Part of the nucleus is left in each of the adjacent sections, where it is likewise distinctly double. Fixed with bichromate-acetic-formalin; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

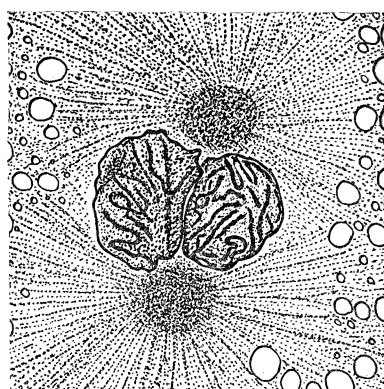
FIG. 36. Horizontal section through a nucleus in the late prophase of the third cleavage mitosis. A line connecting the two asters extends nearly at right angles to the first cleavage furrow. Fixed with bichromate-acetic-formalin; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

FIG. 37. Horizontal section through a daughter-nucleus of a third cleavage mitosis. The two nuclear vesicles remain almost in the position in which they were formed, but two asters are present and these have taken up a position preparatory to the fourth cleavage mitosis. A line connecting the two asters extends in a direction almost parallel to the adjacent newly-formed third cleavage furrow which is vertical and intersects a second furrow almost at right angles. Fixed in bichromate-acetic-formalin; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

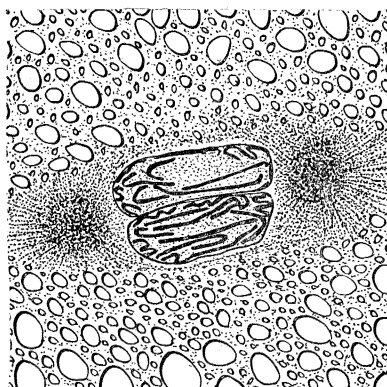
FIG. 38. Horizontal section through a nucleus in position for the fourth cleavage mitosis. A line connecting the two asters extends parallel to the adjacent third cleavage furrow and almost parallel to the first furrow. Fixed in bichromate-acetic-formalin; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.



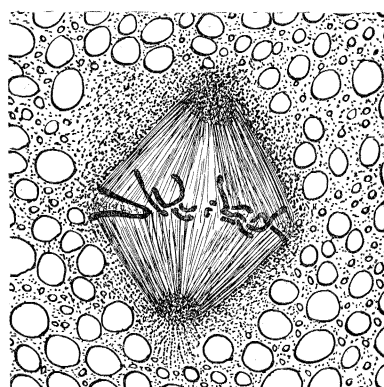
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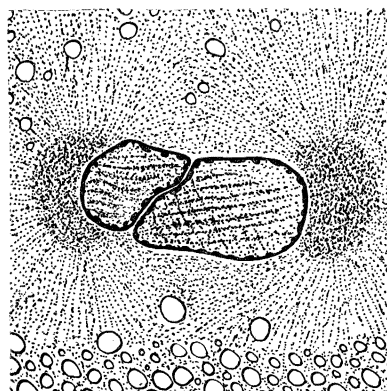
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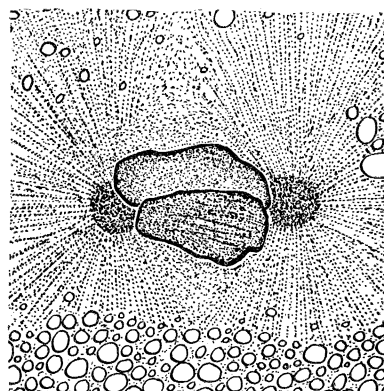
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EXPLANATION OF PLATE VIII.

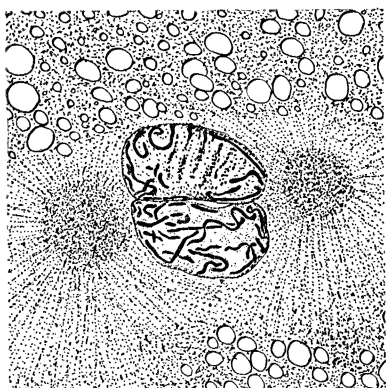
Cryptobranchus allegheniensis.

FIGS. 39 AND 40. Two successive vertical sections through a nucleus preparing for the fourth cleavage mitosis; almost the entire nucleus lies in these two sections. Bichromate-acetic-formalin fixation; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.

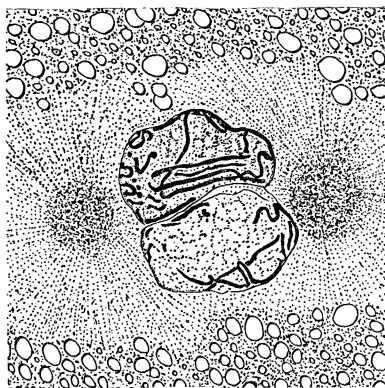
FIG. 41. Another nucleus found in the section used for Fig. 39; early prophase of the fourth cleavage mitosis. $\times 400$.

FIG. 42. Vertical section through a nucleus in the metaphase of the fourth cleavage mitosis. The fourth cleavage furrow has not yet been formed. Bichromate-acetic-formalin fixation; stained with iron-hæmatoxylin. $\times 400$.

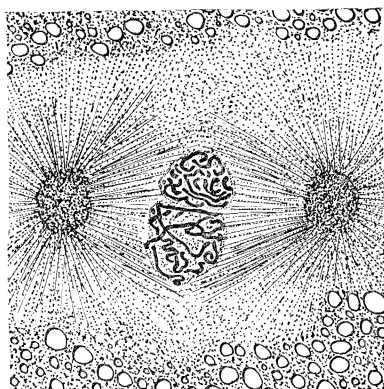
FIGS. 43 AND 44. Two successive horizontal sections through a newly-formed daughter-nucleus of a fourth cleavage mitosis. The fourth cleavage furrow is newly formed. Bichromate-acetic-formalin fixation; stained with borax-carminc Lyons-blue picric-acid. $\times 400$.



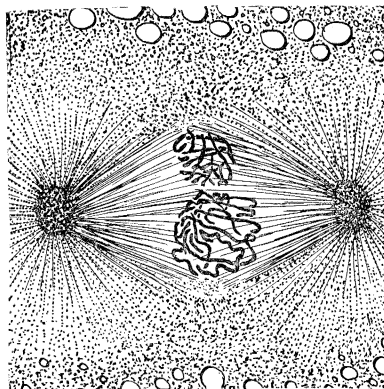
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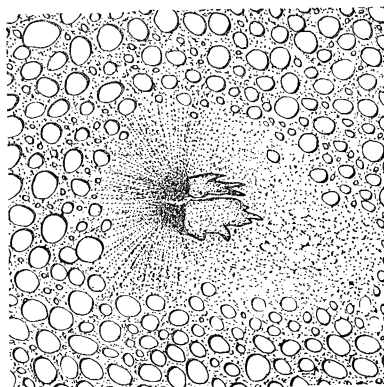
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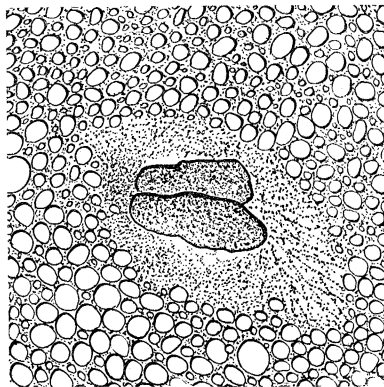
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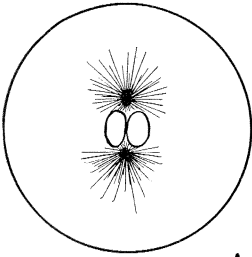
44

EXPLANATION OF PLATE IX.

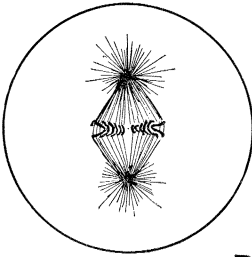
Cryptobranchus alleghehiensis.

FIG. 45. Diagrams illustrating the orientation of the germ-nuclei during the first four cleavage divisions. All the diagrams represent horizontal sections, and the germ-nuclei are assumed to lie always in the same horizontal plane. The size of the nucleus is exaggerated in proportion to the size of the egg. Dotted lines represent beginning cleavage furrows; solid straight lines represent cleavage furrows which have cut to the level of the nuclei. The number of chromosomes formed from each germ-nucleus is assumed to be six, and this is probably the correct number.

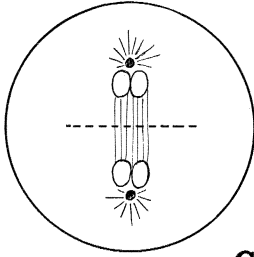
- A. Germ-nuclei and asters in position for the first cleavage mitosis.
- B. First cleavage mitosis.
- C. Newly-formed daughter-nuclei of the first cleavage mitosis.
- D. Nuclear vesicles and asters in position for the second cleavage mitoses.
- E. Second cleavage mitoses.
- F. Newly-formed daughter-nuclei of the second cleavage mitoses.
- G. Nuclear vesicles and asters in position for the third cleavage mitoses.
- H. Third cleavage mitoses.
- I. Newly-formed daughter-nuclei of the third cleavage mitoses.
- J. Nuclear vesicles and asters in position for the fourth cleavage mitoses.
- K. Fourth cleavage mitoses.
- L. Newly-formed daughter-nuclei of the fourth cleavage mitoses.



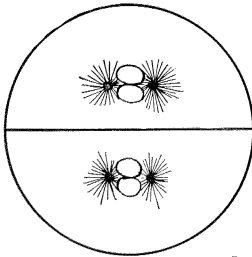
A



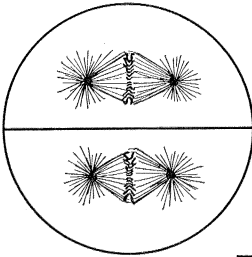
B



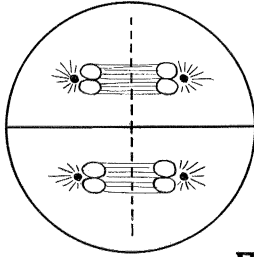
C



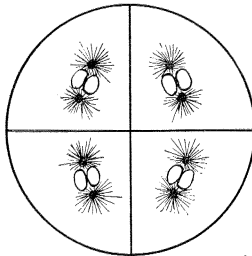
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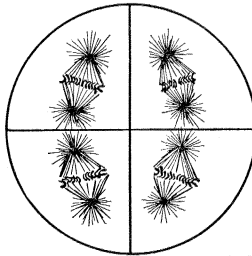
E



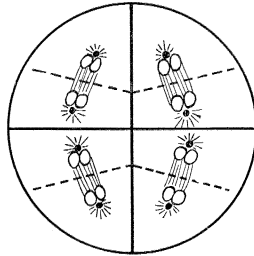
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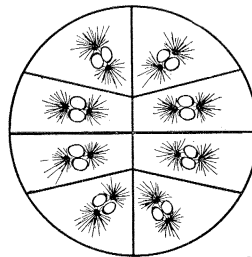
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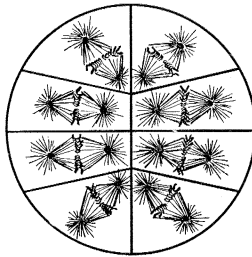
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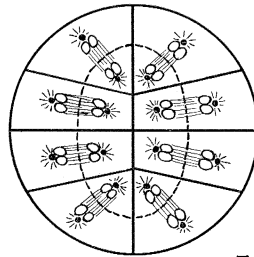
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K



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